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Impacts on soil nitrogen availability of converting managed pine plantation into switchgrass monoculture for bioenergy

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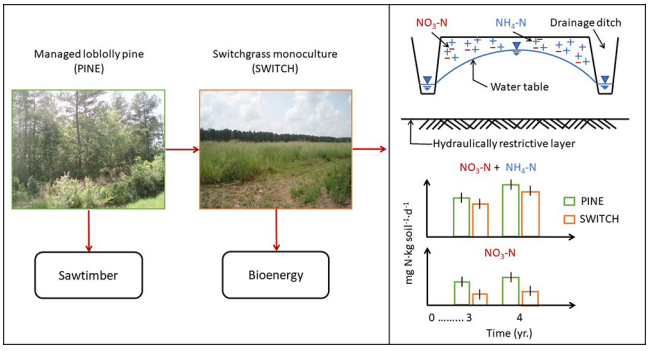
HIGHLIGHTS

- Managed pine (PINE) was converted to switchgrass monoculture (SWITCH) for bioenergy.
- Soil N availability was measured both in the laboratory and in-situ.
- SWITCH did not significantly impact total mineral N availability relative to PINE.
- SWITCH significantly reduced net nitrification rate compared to PINE.

GRAPHICAL ABSTRACT



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ABSTRACT

Biofuels derived from lignocellulosic materials is one of the options in addressing issues on climate change and energy independence. One of the most promising bioenergy crops is switchgrass (*Panicum virgatum* L.), particularly in North America. Future advancement in large-scale conversion of lignocellulosic feedstocks and relatively more competitive price for biomass and other economic advantages could lead to landowners opting to venture on switchgrass monoculture (SWITCH) in lieu of loblolly pine monoculture (PINE). Therefore, we investigated the conversion of previously managed loblolly pine stand into SWITCH in eastern North Carolina, U.S.A. on soil N availability. Treatments included PINE, SWTICH, and mature loblolly pine stand (REF). Each treatment was replicated three times on 0.8 ha plots drained by open ditches dug 1.0–1.2 m deep and spaced at 100 m. Rates of net N mineralization (N_m) and nitrification (N_n) at the top 20 cm were measured using sequential in-situ techniques in 2011 and 2012 (the 3rd and 4th years of establishment, respectively) along with a one-time laboratory incubation. On average, PINE, SWITCH, and REF can have field net N_m rates up to 0.40, 0.34 and 0.44 mg N·kg soil^{-1}·d^{-1}, respectively, and net N_n rates up to 0.14, 0.08 and 0.10 mg N·kg soil^{-1}·d^{-1}, respectively. Annually, net N_m rates ranged from 136.98 to 167.21, 62.00 to 142.61, and 63.57 to 127.95 kg N·ha^{-1}, and net N_n rates were 56.31–62.98, 16.45–30.45, 31.99–32.94 kg N·ha^{-1} in PINE, SWITCH, and REF, respectively. Treatment effect was not significant on field N_m rate ($p = 0.091$). However, SWITCH significantly reduced

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nitrate-N production ($p < 0.01$). Overall, results indicated that establishment of SWITCH on poorly drained lands previously under PINE is less likely to significantly impact total soil N availability and potentially has minimum N leaching losses since soil mineral N under this system will be dominated by ammonium-N.

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1. Introduction

Lignocellulosic-based or second-generation biofuel is one of the options in response to increasing concerns on climate change and energy independence. Among the most promising bioenergy crops is switchgrass (*Panicum virgatum* L.) (Vogel, 1996; McLaughlin and Kszos, 2005; Wright and Turhollow, 2010; Jones, 2013), a perennial warm-season grass commonly found in the tall prairies of North America (Vogel et al., 2002). In the southeastern U.S., loblolly pine-switchgrass intercropping (PSWITCH) has already been explored to simultaneously produce sawtimber and bioenergy feedstock (Blazier, 2009; Chescheir et al., 2011). Susaeta et al. (2012) showed that based on an annual switchgrass yield of 12 Mg ha^{-1}, landowners will profit more with traditional loblolly pine (PINE) at a switchgrass price of <$30 Mg^{-1}, but they will earn more with PSWITCH at a switchgrass price ≥$30 Mg^{-1}. Favorable incentives through the Farm Bill of 2008 (Susaeta et al., 2012) and as technologies for converting biomass to liquid fuel advance, switchgrass production for biofuel may become economically competitive which could lead to conversion of a large portion of managed pine to switchgrass monoculture. A fundamental understanding of its impact on soil N cycling and availability is critical for developing and implementing best management practices for this system.

Assuming switchgrass prices become competitive, there are two main driving factors that landowners may opt to choose switchgrass monoculture (SWITCH) in lieu of PINE or PSWITCH. One factor is, SWITCH has a very short waiting time for income generation relative to PINE. While it takes 25 years (average rotation time) to harvest loblolly pine trees, switchgrass can be harvested after the first growing season with maximum productivity attainable within 3–4 years from establishment (Parrish and Fike, 2005) and does not require additional site preparation due to its perennial nature. Another factor is, switchgrass has longer productive years and greater yield under SWITCH than PSWITCH. The effective life-span of switchgrass under monoculture can reach over 15 years (Rinehart, 2006). Under PSWITCH with tree-bed spacing of 6.1 m, switchgrass production is reduced to approximately 30% (Tian et al., 2015) and will likely cease after 7–8 years from establishment as tree canopy closes.

Like many terrestrial ecosystems, nitrogen (N) is the most critical nutrient limiting the productivity of southeastern U.S. forests (Pritchett and Smith, 1975). As a managed system, N becomes available to loblolly pine trees through N mineralization and fertilization. Nitrogen mineralization, a microbially-mediated process, is the conversion of organic nitrogen into mineral N compounds (e.g., NH_4^+-N and NO_3^--N) and is influenced by common silvicultural management practices including site preparation (Fox et al., 1986; Vitousek et al., 1992), herbicide application (Smethurst and Nambiar, 1989; Woods et al., 1992; Gurlevik et al., 2004), fertilization (Gurlevik et al., 2004), and harvesting (Likens et al., 1970). Understanding changes in soil N availability due to land cover change together with the knowledge of plant nutrient requirement is critical for ensuring maximum productivity and minimizing water quality impacts.

Soil N mineralization and nitrification processes are primarily linked with carbon (C) dynamics in both forested (Melillo et al., 1989; Zak et al., 1993; Hart et al., 1994; Scott and Binkley, 1997; Finzi et al., 1998; Booth et al., 2005) and grassland (Elliott, 1986; Schimel, 1986; Barrett and Burke, 2000; Booth et al., 2005) ecosystems during the decomposition of organic matter. Conversion of managed loblolly pine to switchgrass monoculture could impact soil N cycling due to associated changes in soil C quantity and/or quality. Previous research showed that switchgrass produce large belowground biomass, particularly fine-roots (Garten and Wullschleger, 1999; Ma et al., 2000a; Frank et al., 2004; McLaughlin and Kszos, 2005; Garten Jr. et al., 2010). Further, switchgrass monoculture established in degraded lands or lands previously under conventional annual crop production has been found to increase soil organic C pools and particulate organic matter fractions (Ma et al., 2000b; Frank et al., 2004; Liebig et al., 2005; McLaughlin and Kszos, 2005; Dou et al., 2013), and mineralizable and microbial biomass C (Ma et al., 2000c; Chatterjee et al., 2013; Dou et al., 2013). Some forested sites experienced decline in surface C when invaded by grass species (e.g., Strickland et al., 2010, 2011), while others did not (Kourtev et al., 2003; Litton et al., 2006). However, soil C stocks in native forests of southeastern U.S. generally decline due to grass invasion (Strickland et al., 2010, 2011; Kramer et al., 2012). In the same region, Strickland et al. (2015) also found significant net reduction in soil C stocks on the third year of switchgrass intercropped with managed loblolly pine and grown in monoculture. They argued that C inputs from switchgrass which is potentially more labile relative to the native vegetation (Blazier et al., 2012) triggered a priming effect (Blagodatskaya and Kuzyakov, 2008) which has been associated with net soil C loss (Fontaine et al., 2004; Bradford et al., 2008).

Establishment of switchgrass monoculture for bioenergy on a formerly row crop dominated system altered N cycling and availability (Smith et al., 2013). Similarly, switchgrass intercropped with managed loblolly pine stand significantly reduced net nitrification rate, but did not significantly affect net N mineralization rate and its temporal variations (Cacho et al., 2018a). Impacts of converting managed forests into grass monoculture for bioenergy on net N mineralization and nitrification have not been widely explored as opposed to forest conversion to pasture (e.g., Piccolo et al., 1994; Reiners et al., 1994; Neill et al., 1997). Therefore, we studied impacts of growing switchgrass monoculture for biofuel under previously managed loblolly pine stand in eastern North Carolina on net N mineralization and nitrification in years three and four, respectively, which are the expected years when switchgrass will reach maximum productivity potential. We used measured net N mineralization and nitrification rates multiple times within each year using sequential in-situ technique (Raison et al., 1987; Robertson et al., 1999) and a one-time laboratory incubation.

2. Materials and methods

2.1. Study site and experimental treatments

This study was conducted near Dover, Lenoir County, North Carolina, U.S.A. (35° 15′ N, 77° 27′ W) (Fig. 1a and b). The site was owned and managed by Weyerhaeuser Company. Its detailed description including soil series classification, textural classes, etc. can be found elsewhere (e.g. Beauvais, 2010; Albaugh et al., 2012; Cacho et al., 2015). Two management regimes were analyzed in this study including traditional pine with biomass left in place (PINE) (Cacho et al., 2015) and switchgrass only (SWITCH) plots (Fig. 1c). Additionally, a mature loblolly pine stand established in 1974 (REF) (Fig. 1c) was included for qualitative comparison. Trees in PINE and REF were established using 6.1 × 1.5 m and 2.65 × 1.5 m spacing schemes, respectively (Cacho et al., 2015). Establishment of PINE was conducted on plots with non-merchantable materials left in place after harvesting of the old loblolly pine stand. Plots for SWITCH were more intensively prepared for successful

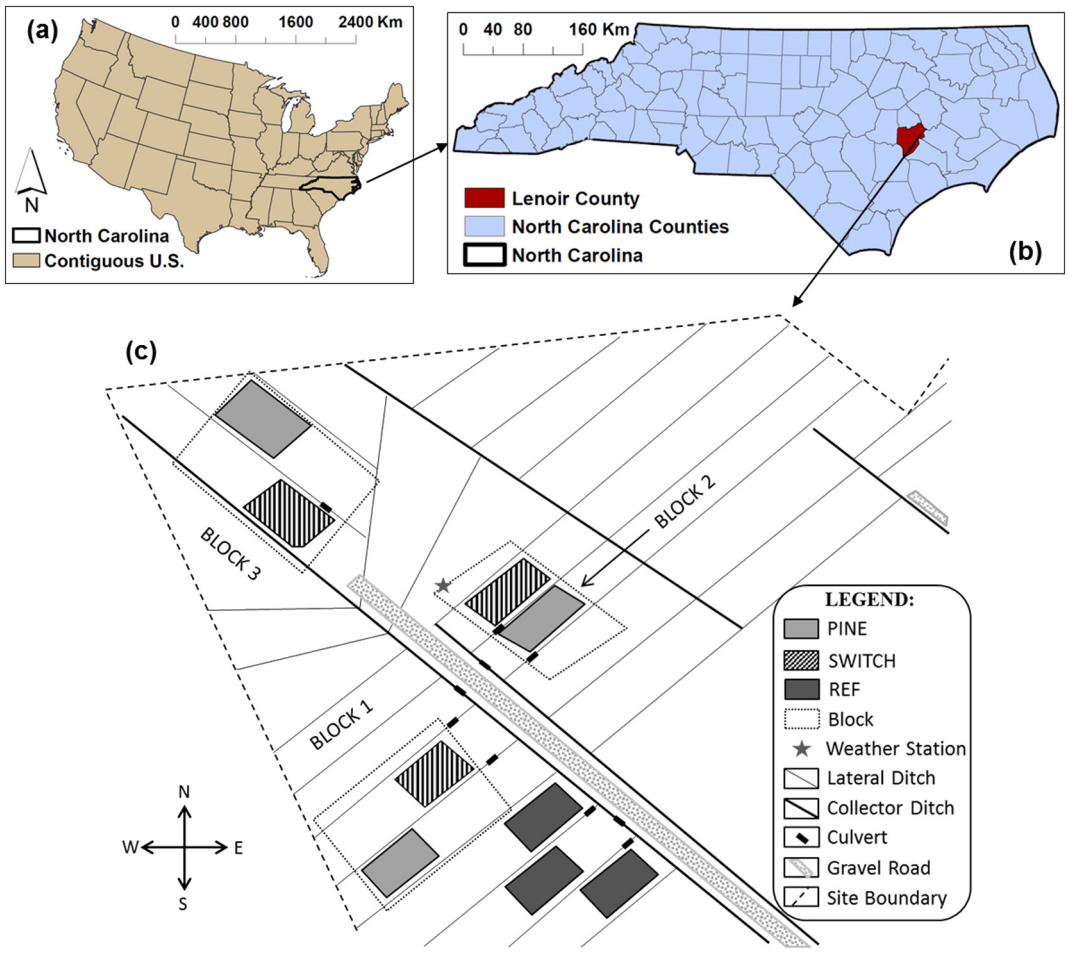

Fig. 1. The study site; (a) contiguous United States, (b) State of North Carolina, and (c) schematic of experimental treatments (not drawn to scale). Modified from Cacho et al. (2018a).

establishment which included harvest residue removal and root raking (Table 1). Each management regime or treatment was replicated three times on 0.8 ha plots (Fig. 1) drained by parallel open ditches dug 1.0–1.2 m deep and spaced at approximately 100 m.

Table 1
Timeline of management practices implemented on each management regime. (Adapted and modified from Cacho et al., 2018b)

Management practice	Time	Management regimes		
		PINE	SWITCH	REF
Clearcut harvesting of previous stand	9/2008	✓	✓	
Site preparation and V-shearing	10/2008	✓	✓	
Root raking	11/2008		✓	
Aerial herbicide application	11/2008	✓	✓	
Bedding & pre-plant fertilizer application	11/2008	✓	✓	
Planting of pine seedlings	12/2008	✓	✓	
Herbicide application	5/2009		✓	
V-shearing streaks for switchgrass planting	5/2009		✓	
Planting of switchgrass	6/2009		✓	
1st switchgrass harvesting[a]	3/2010		✓	
Coated Arborite®fertilizer application	6/2010		✓	
Herbicide application (2, 4-D and Basagran)	6/2010		✓	
2nd switchgrass harvesting[b]	12/2010		✓	
3rd switchgrass harvesting[b]	12/2011		✓	
Coated Arborite®fertilizer application	4/2012		✓	

"✓" denotes implementation of a management practice.
[a] Switchgrass was mowed and retained on site.
[b] Switchgrass was mowed, raked, baled and removed off treatments.

2.2. Data collection

2.2.1. Field incubation

Field measurements of net N mineralization (N_m) and nitrification (N_n) rates were conducted using sequential in-situ incubation technique (Raison et al., 1987; Robertson et al., 1999). Soil sample collection and incubation were performed within a 30 × 40 m subplot at the center of each plot (Fig. 2a and d) using a thin-walled polyvinyl chloride (PVC) pipes (Fig. 2e). For PINE and REF soil samples were incubated and collected from three sampling points including near a tree (NT, approx. 20 cm from the tree trunk), at the midpoint between two trees on the same bed (B2T) and at the center point of four trees on two adjacent beds (M4T) (Fig. 2a). Due to the absence of tree beds in SWITCH, soil collection and incubation were done at three locations within the 30 × 40 m subplot including at plot center (OC), at 15 m near plot center (NC) and 30 m from plot center (Fig. 2d). In all treatments, each sampling point within each subplot was replicated three times. During each incubation period, two PVC pipes within 10–15 cm horizontally on centers of each other were hand-driven using a rubber mallet to a depth of 20 cm (Fig. 2e). One of the two PVC pipes was taken out immediately, while the other was left in the field for 28 days. Soil samples from PVC pipes that were immediately removed were processed in the laboratory and analyzed for initial ammonium-N (NH_4^+N) and nitrate-N (NO_3^-N) concentrations. Similarly, soil samples from PVC pipes left in the field were collected after 28 days, which then were processed and analyzed for final ammonium-N (NH_4^+N) and nitrate-N (NO_3^-N), following the same procedures as

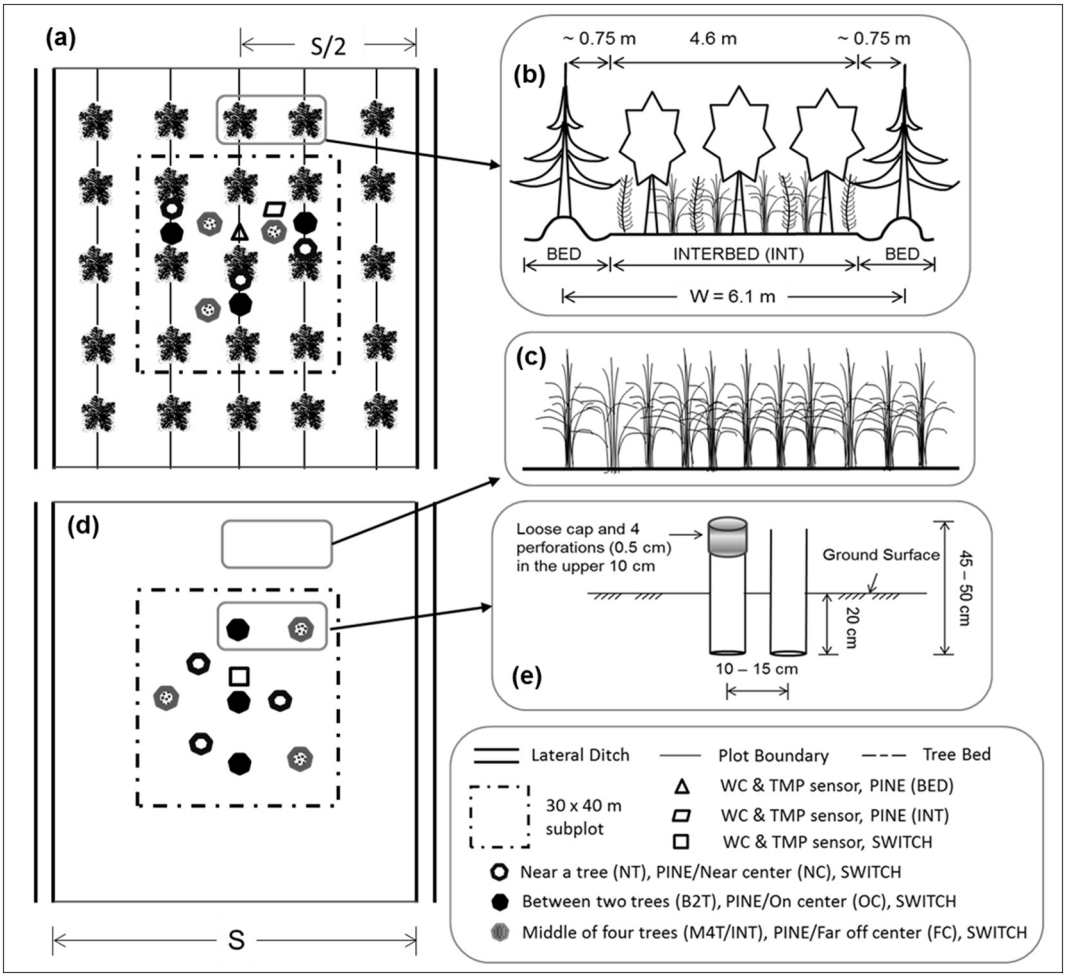

Fig. 2. Schematic of soil sample collection points and related measurements (not drawn to scale) in (a) traditional pine stand (PINE) and REF, and (d) switchgrass only plots where (b) and (c) are detailed illustration of vegetation growing in each treatment, and (e) detailed description of incubation pipes.
Adapted and modified from Cacho et al. (2018a).

the initial samples. The reader is referred elsewhere (e.g., Cacho, 2013; Cacho et al., 2018a) for detailed description of PVC pipe set-up in the field, soil sample processing, and extraction of inorganic N. Rates of net N mineralization (N_m) and nitrification (N_n) were computed using Eqs. (1) and (2), respectively (Robertson et al., 1999). Relative nitrification (N_R) was calculated using Eq. (3), which was based on the inorganic N concentrations at the end of incubation period (Robertson, 1982).

$$N_m = \frac{\left(\left[NH_4^+N\right] + \left[NO_3^-N\right]\right)_f - \left(\left[NH_4^+N\right] + \left[NO_3^-N\right]\right)_i}{T} \quad (1)$$

$$N_n = \frac{\left(\left[NO_3^-N\right]\right)_f - \left(\left[NO_3^-N\right]\right)_i}{T} \quad (2)$$

$$N_R = \frac{\left[NO_3^-N\right]_f}{\left(\left[NH_4^+N\right]_f + \left[NO_3^-N\right]_i\right)} \times 100 \quad (3)$$

where; N_m = net N mineralization rate ($mg \cdot N \cdot kg^{-1} \cdot d^{-1}$); N_n = net nitrification rate ($mg \cdot NO_3^-N \cdot kg^{-1} \cdot d^{-1}$); N_R = relative nitrification (%); $[NH_4^+N]_f$ and $[NH_4^+N]_i$ = final and initial ammonium-N concentrations, respectively; $[NO_3^-N]_f$ and $[NO_3^-N]_i$ = final and initial nitrate-N concentrations, respectively; and T = incubation time (28 days).

Soil physical and chemical properties including bulk density (ρ_b), pH, total carbon (TC) and total nitrogen (TN) contents were measured in 2011. Details of the methods of processing and analyzing soil samples for these properties can be found in Cacho (2013) and Cacho et al. (2018a). Field soil water content and temperature were measured at 10 cm depth at each plot center using dedicated sensors and dataloggers. Description of the sensors and dataloggers used has been mentioned elsewhere (e.g., Tian et al., 2016). In situ soil water (SW) content reported here were values at the beginning of each incubation, while soil temperature (TMP) values represented were average over the 28-day incubation period. Soil water content and temperature data for REF represented values in the BED only since there was no measurement of these parameters being conducted in the INT.

2.2.2. Laboratory incubation

To complement our in-situ net N_m and N_n measurements, a one-time laboratory incubation was conducted. These soil samples were taken from the same sampling points in each treatment and approximately within 50 cm of the location of field incubated soils. Samples for laboratory incubation were processed and handled the same way as field incubated samples and composited by location. The primary purpose of laboratory incubation was to subject soil samples to optimum SW content (60% of water filled pore space or WFPS) and temperature (25 °C) so that decomposition becomes substrate limited. This enabled us to answer whether differences in litter quality between treatments have impacts on net N_m and N_n.

Background concentrations of NH_4^+N and NO_3^-N of each composite sample were determined from triplicate 10 g subsamples. A 50 g subsample was also taken from each composite sample and dried at

105 °C for 24 h to determine initial SW contents of the samples. Values of initial SW content and ρ_b were used to compute the amount of water needed to raise SW content of each composite sample to 60% WFPS using Eq. (5), which was a result of rearranging Eq. (4) (Robertson et al., 1999). Triplicates of 10 g from each composite sample were prepared and placed inside 100 mL specimen container. Based on the results of computation using Eq. (5), distilled water was carefully added to the 10 g subsamples inside specimen container using a small water dropper to raise their respective SW contents to 60% WFPS. Each specimen container was then covered with a lid having five 0.16 cm perforations to ensure proper aeration and placed in an incubator with a constant temperature of 25 °C. Further, a small water bowl filled with distilled water was placed at the bottom of the incubator to minimize, if not prevent water loss from the specimen container. Nevertheless, each sample was checked once a week for water loss and distilled water was added as necessary. Soil samples were incubated for one-, two-, eight- and thirteen weeks. Soil inorganic nitrogen contents were extracted and analyzed following the same procedures as field incubated samples. Like field incubated samples, laboratory net N_m and N_n rates, and N_R were computed using Eqs. (1), (2) and (3), respectively.

$$\%WFPS = \left[\theta_m\left(\frac{\rho_b}{n_o}\right)\right]100 \tag{4}$$

where; $\%WFPS$ = percent water-filled pore space; θ_m = gravimetric soil water content ($g\ H_2O \cdot g\ dry\ soil^{-1}$); ρ_b = bulk density ($g \cdot cm^{-3}$); n_o = total porosity (%).

$$gH_2O = \left[\left(\frac{\%WFPS_o}{100}\right)\left(\frac{n_o}{\rho_b}\right)m_s\right] \tag{5}$$

where; gH_2O = amount of deionized water required by each subsample (g) to reach 60% $WFPS$; $\%WFPS_o$ = optimum water-filled pore space set at 60%; m_s = mass of subsample which was set at 10 g.

2.3. Statistical analysis

Data from reference or mature loblolly pine stand (REF) were excluded from statistical tests since it was not randomly assigned to each of the three blocks and used for qualitative comparison only. Data from PINE and REF were adjusted by the relative importance of the areas covered by tree bed (BED) and interbed (INT) to the entire plot using Eqs. (6) and (7), respectively. Although both treatments have the same spacing of trees within a BED (1.5 m), spacing between tree beds was wider in PINE (6.1) m (Fig. 2b) than REF (2.65 m). As such, BED and INT cover approximately 25% and 75%, respectively in PINE (Fig. 2b). Conversely, BED and INT constitute 55% and 45%, respectively in REF.

$$Y = \frac{(0.25 * \rho_{b,BED} * X_{BED}) + (0.75 * \rho_{b,INT} * X_{INT})}{(0.25 * \rho_{b,BED} + 0.75 * \rho_{b,INT})} \tag{6}$$

where; Y = adjusted value of a parameter (e.g. N_m, N_n, N_R, etc.); $\rho_{b,\ BED}$ and $\rho_{b,\ INT}$ = bulk density values in the BED and INT, respectively; and X_{BED} and X_{INT} = unadjusted parameter values in the BED and INT, respectively in PINE.

$$Z = \frac{(0.25 * \rho_{b,BED} * X_{BED}) + (0.75 * \rho_{b,INT} * X_{INT})}{(0.25 * \rho_{b,BED} + 0.75 * \rho_{b,INT})} \tag{7}$$

where; Z = adjusted value of a parameter (e.g. N_m, N_n, N_R, etc.); $\rho_{b,\ BED}$ and $\rho_{b,\ INT}$ = bulk density values in the BED and INT, respectively; and X_{BED} and X_{INT} = unadjusted parameter values in the BED and INT, respectively in REF.

Data analyses were performed using analysis of variance (ANOVA) for one-time (e.g. ρ_b, pH, TC, TN, and C/N) and repeatedly (e.g. N_m, N_n, N_R, SW, and TMP) measured parameters. Two models were used for repeated measures ANOVA with time of incubation as repeated measures factor. Model 1 was used for analyzing laboratory results with treatment, time and treatment × time as fixed effect factors and block as random effect factor. Model 2 was used to analyze field results with treatment, year, treatment × year, time (year), treatment × time (year) as fixed effect factors and block, block × treatment and block × treatment × year as random effect factors. Multiple covariate structures including compound symmetric (CS), compound symmetric heterogeneous (CSH), unstructured (UN), unstructured first-order (UN(1)), ante-dependence first-order (ANTE(1)) and spatial power (sp(pow)) were tested and compared. Selection of CV structure was based on best model fit statistics (e.g. lower values of log likelihood ratio, AIC, AICC, BIC or SBC, CAIC and HQIC (SAS Institute, Inc., 2012)). Data transformations were conducted as necessary to satisfy assumptions of parametric statistical tests. Data analyses were carried out using mixed procedures (PROC Mixed and GLIMMIX) in SAS 9.4 software package. Tests of type III fixed effects and pairwise mean differences comparisons using Tukey-Kramer adjustment were based on probability level (α) of 0.05. Moreover, $\alpha = 0.05$ was used for testing treatment mean differences at each incubation time. Values of the mean and standard error in REF were determined arithmetically for qualitative comparisons with tested management regimes or treatment.

3. Results

3.1. Soil physical and chemical properties, and environmental factors

Mean bulk density density (ρ_b) in SWITCH (1.04 $g \cdot cm^{-3}$) was higher than PINE and REF (<0.95 $g \cdot cm^{-3}$) (Table A.1). However, the difference in mean ρ_b between SWITCH and PINE was not significant ($p = 0.072$). Mean pH value in SWITCH (4.2) was also higher than PINE and REF (<4.02) (Table A.1). Like (ρ_b), treatment effect on pH was not significant ($p = 0.071$). Mean values of total C (TC) content, total N (TN) content, and carbon-to-nitrogen (C:N) ratio in SWITCH were consistently lower relative to PINE, but none of the differences were significant (Table A.2).

It should be noted that mean soil water (SW) content and soil temperature (TMP) data for REF were values of in the BED only (REF-BED in Fig. A.1). Mean SW contents across treatments were affected by year ($p = 0.018$) with 2012 tending to have higher values (Fig. 3b). They were not affected by time within year (Table A.3), but lower values were usually observed during the growing season (Fig. 3c). Mean SW content in SWITCH tended to be higher compared to PINE and REF (Fig. A.1a, b, and c). Difference in mean SW content between SWITCH and PINE was significant ($p = 0.02$). Like mean SW content, mean soil temperatures (TMP) across treatments were affected by year ($p = 0.001$) with 2011 having higher values (Fig. 3e). They were also affected by time within year (Table A.3). Mean peak values consistently occurred during July–August in both years (Fig. A.1f). Treatment effect on mean TMP was not significant (Table A.3).

3.2. In-situ net N mineralization and nitrification rates, and relative nitrification

In-situ net N mineralization (N_m) rates were significantly affected by year ($p = 0.048$) with 2012 having higher N_m rates across treatments. Among treatments, PINE tended to have higher net N_m rate. Overall, mean N_m rates were 0.23-, 0.13- and 0.14 mg $N \cdot kg\ soil^{-1} \cdot d^{-1}$ in PINE, SWITCH and REF, respectively (Fig. 3a). In 2011, PINE had mean N_m rate of 0.21 mg $N \cdot kg\ soil^{-1} \cdot d^{-1}$, while SWITCH and REF had 0.08- and 0.09 mg $N \cdot kg\ soil^{-1} \cdot d^{-1}$, respectively (Fig. 3b). Similarly in 2012, PINE had mean N_m rate of 0.26 mg $N \cdot kg\ soil^{-1} \cdot d^{-1}$, while SWITCH and REF had 0.19- and 0.18 mg $N \cdot kg\ soil^{-1} \cdot d^{-1}$, respectively (Fig. 3b). Annual areal equivalent of these rates are summarized in Table 4. Differences in mean N_m rates between PINE and SWITCH both overall and on annual basis were not significant (Table 3). A significant

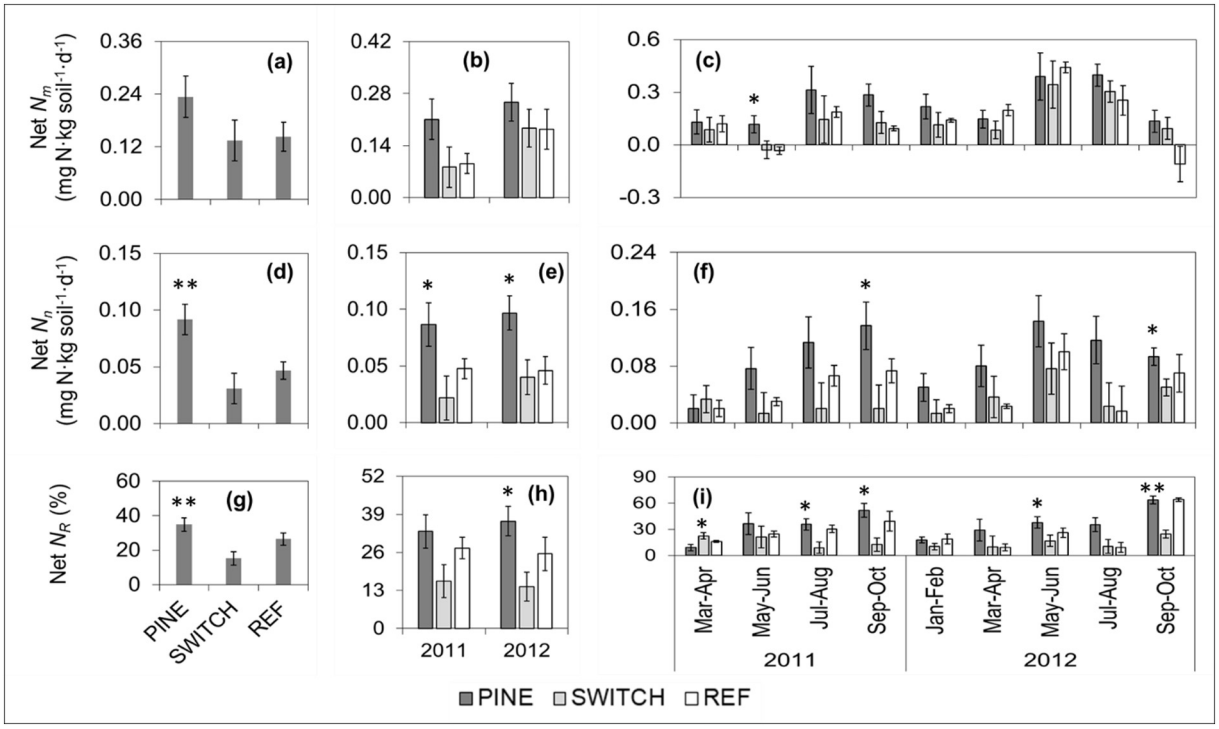

Fig. 3. In situ rates (mg N·kg soil^{-1}·d^{-1}) of net N mineralization (N_m) and nitrification (N_n), and relative nitrification (N_R) (%). Left-hand (a, d and g), middle (b, e and h) and right-hand (c, f and i) column panels were mean N_m, N_n and N_R averaged over the entire study period, annually and at each incubation period, respectively. Means marked with * and ** were significantly higher at $\alpha = 0.05$ and $\alpha = 0.01$, respectively.

effect of time within year or time (year) (Table 3) on mean N_m rates was found with values tending to be higher in the growing season (Fig. 3c). Peak mean N_m rates in the growing season could go up to 0.4-, 0.34- and 0.44 mg N·kg soil^{-1}·d^{-1} in PINE, SWITCH and REF, respectively (Table 2).

Unlike N_m, mean net nitrification (N_n) rates were not significantly affected by year ($p = 0.389$). Mean N_n rates in SWITCH and REF were comparable, but tended to be lower than PINE (Fig. 3d, e and f). Overall, mean N_n rates in PINE, SWITCH and REF were 0.09-, 0.03-, and 0.05 mg N·kg soil^{-1}·d^{-1}, respectively (Fig. 3d). In 2011, the average rates of N_n were 0.09-, 0.02- and 0.05 in PINE, SWITCH and REF, respectively (Fig. 3e). Similarly in 2012, mean N_n rates were 0.1- and 0.04 mg N·kg soil^{-1}·d^{-1} in PINE and SWITCH, respectively, while in REF, mean N_n rate stayed at 0.05 mg N·kg soil^{-1}·d^{-1} (Fig. 3e). Areal equivalent of these N_n rates in all treatments can be found in Table 4. The overall decrease in the mean N_n in SWITCH was significantly different than PINE ($p = 0.006$). None of the other fixed effects had significant effects on mean N_n rate (Table 3). In general, peak mean N_n rates in PINE and REF which could reach up to 0.14- and 0.10 mg N·kg soil^{-1}·d^{-1}, respectively (Table 2), occurred between early summer and early fall across years, while in SWITCH its timing varied from year to year (Fig. 3f). In 2011, peak mean N_n rate in March–April at 0.03 mg N·kg soil^{-1}·d^{-1}, while in 2012 it occurred in May–June at 0.08 mg N·kg soil^{-1}·d^{-1} (Fig. 3f; Table 2).

Consistent with N_n rates, mean relative nitrification (N_R) was not significantly affected by year ($p = 0.879$). Further, mean N_R tended to be highest and lowest in PINE and SWITCH, respectively (Fig. 3g, h and i). Overall, values of mean N_R in PINE, REF and SWITCH were 34.92-, 26.41- and 15.24%, respectively (Fig. 3g). In 2011, mean N_R values in PINE, REF and SWITCH were 33.17-, 27.5- and 16.18%, respectively, while in 2012 they were 36.67-, 25.53- and 14.3%, respectively (Fig. 3h). Overall, the difference in mean N_R between PINE and SWITCH was significant ($p = 0.005$). A significant time within year effect (time (year)) on mean N_R was also significant ($p = 0.001$) with higher values tending to occur between early summer and early fall, particularly for PINE (Fig. 3i). Peak values of mean N_R for PINE and REF which could be up to 63.6- and 64%, respectively (Table 2) usually occurred in early fall across years. Conversely, in SWITCH which could be up to 24.4% (Table 2) varied from early spring in 2011 to early fall in 2012 (Fig. 3i). A significant treat × time within year interaction effect on mean N_R was significant (Table 3) where significant differences between PINE and SWITCH usually occurred during summer and early fall, respectively (Fig. 3i).

3.3. Laboratory net N mineralization and nitrification rates, and relative nitrification

Laboratory mean net N mineralization (N_m) rates were comparable among treatments (Fig. 4a). Highest and lowest mean N_m rates across treatments occurred consistently during the 1st and 13th week, respectively (Fig. 4b). There were no significant effects of treatment, time, and treatment × time interaction on N_m (Table 5). Mean net nitrification

Table 2
Range of mean net N mineralization (N_m) and nitrification (N_n) rates (mg N·kg soil^{-1}·d^{-1}), and relative nitrification (N_R) (%) in situ in 2011 and 2012 for all treatments.

Treatment	N_m		N_n		N_R	
	2011	2012	2011	2012	2011	2012
PINE	0.12–0.31	0.13–0.4	0.02–0.14	0.05–0.14	9–51.67	17.67–63.67
SWITCH	−0.03–0.14	0.08–0.34	0.01–0.03	0.01–0.08	8.77–22.43	9.77–24.43
REF	−0.03–0.19	−0.11–0.44	0.02–0.07	0.02–0.1	16–39	9.33–64

Table 3
Results (p-values) of type III test of fixed effects ANOVA of net N mineralization (N_m) and net nitrification (N_n) rates, and relative nitrification (N_R) from in-situ incubation.

Source	DF	N_m	N_n	N_R
Treat	1	0.091	0.006	0.005
Year	1	0.048	0.389	0.879
Treat * year	1	0.408	0.795	0.616
Time (year)	7	0.001	0.075	0.001
Treat * time (year)	7	0.968	0.531	0.015

(N_n) rate was highest in PINE (4c). However, difference in mean net N_n rates between PINE and SWITCH was not significantly different ($p = 0.061$). Occurrences of highest and lowest N_n rates across treatments (Fig. 4d) tended to follow similar timing as N_m (Fig. 4b). No significant effects of time and treat × time interaction on mean N_n rate (Table 5). Relative nitrification (N_R) were highest and lowest in PINE and REF, respectively (Fig. 4e and f). Mean peak N_R values tended to occur in the later part of the incubation periods (Fig. 4f). None of the fixed effects including treatment, time, and treatment × time interaction were significant (Table 5).

4. Discussion

4.1. Soil physico-chemical properties and environmental factors

Relatively more intensive management regimes for SWITCH, particularly root raking and the extra V-shearing operation (Table 1) could explain why it had higher mean ρ_b than PINE and REF. Mean values of pH across treatments were consistent with the Pantego and Rains soil series (Barnhill, 1977) and comparable to a pH value reported in an earlier study conducted on the same site (e.g. Albaugh et al., 2014). Mean values of total carbon (TC) and total nitrogen (TN) contents, carbon-to-nitrogen (C:N) ratio across treatments were within range of values reported by another study conducted on our site (e.g. Minick et al., 2015). Significantly higher soil water (SW) content in SWITCH relative to PINE was not surprising knowing that it has lower transpiration rate (Albaugh et al., 2014) and consistently shallower water table depth (unpublished data). Seasonal variations of mean soil temperature across treatments (Fig. A.1) were expected and self-explanatory.

4.2. Impacts of litter quality, environmental factors and management on net N mineralization (N_m) and nitrification (N_n) rates

Different plant species vary in timing of litter inputs, and litter quantity and quality, which can affect organic matter decomposition rates (Berendse et al., 1989; Melillo et al., 1982; Seastedt, 1988). Significant differences in N cycling and dynamics were observed even within grasses grown in monoculture systems relative to their photosynthetic pathways (C3 vs. C4) (Wedin and Tilman, 1990). By subjecting soil samples in the laboratory at optimum environmental conditions, mineralization of organic N becomes substrate limited (Stanford and Smith, 1972; Paul and Clark, 1996). In so doing, we were able to isolate the effect of presumably varying litter quality among treatments on soil N transformations. Laboratory results showed that changes in litter quality due to planting of switchgrass (a C4 species) that has a potentially more labile litter inputs than a C3-dominated native vegetation (Blazier et al., 2012) did not significantly affect net N mineralization (N_m) ($p = 0.363$) and nitrification (N_n) ($p = 0.061$) rates. Results of previous studies on impacts of converting forests into grass monoculture (e.g., pasture) on soil N transformations were divergent. For instance, Luizao et al. (1992) did not find significant difference on an annual cycle between net N_m and N_n rates in a tropical forest and a 1-year old pasture. Further, a one-time measurement by Matson et al.

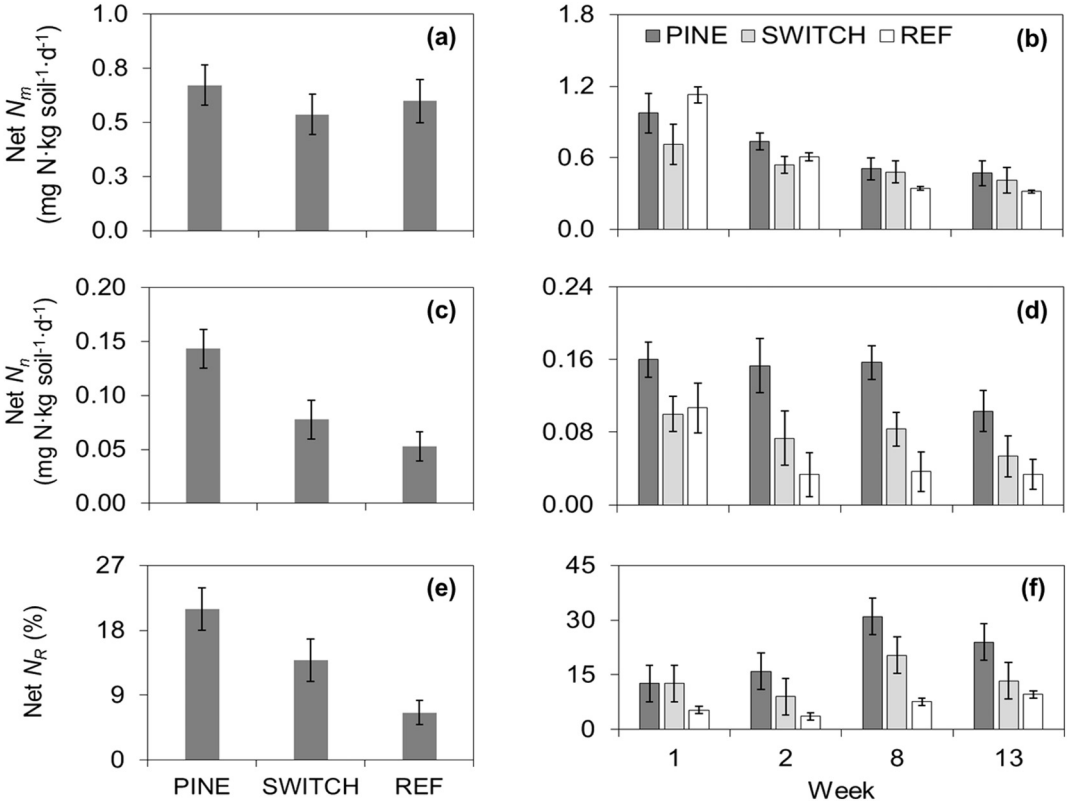

Fig. 4. Laboratory rates of net N mineralization (N_m) (a and b) and nitrification (N_n) (c and d), and relative nitrification (N_R) (e and f). Left column panels are rates of N_m (a) and N_n (c), and N_R averaged over 13 weeks. Error bar represents standard error of the mean.

(1990) in a forest and a 3-year old pasture showed similar pattern. Conversely, Neill et al. (1997) found net N_m and N_n rates to be consistently lower in a 3-year pasture planted on cleared forest site than in original forest. This suggests that rates of net N_m and N_n in grass monoculture established on previously forested sites are not primarily governed by litter quality and/or quantity. Temporal patterns of laboratory N_m were expected. Rates of N_m were highest in the earlier periods of incubation as supply of labile substrate was abundant, but lowest during the later periods as substrate became predominantly recalcitrant. Higher proportions of total inorganic nitrogen that were nitrate or relative nitrification (N_R) in the later part of the incubation periods (Fig. 4f) were not surprising knowing that these were also periods when ammonium N accumulation was expected to be at its peak.

The significant increase in field rates of N_m from 2011 to 2012 across treatments can be related to significant inter-annual changes in environmental factors, particularly soil water (SW) content (Fig. A.1b). A much drier growing season in 2011 (Fig. A.1c) may have significantly affected N_m rates across treatments in 2011. It could be argued that priming effect (Kuzyakov et al., 2000) had likely contributed to the overall increase in N_m rates since fertilization was applied in 2012, but not in 2011 in SWITCH. However, N_m rates increase in both PINE and REF which did not receive fertilization in 2012 suggests that this was not the case.

The total mineral N production in the field and its temporal variations were not significantly affected by SWITCH ($p = 0.091$) despite having a more intensive site preparation and other management operations (Table 1). This could be primarily explained by the presence of a more active microbial biomass in switchgrass introduced to a forested system (Strickland et al., 2015). On the other hand, SWITCH significantly impacted the production of NO_3^--N (Fig. 3d–i), which is consistent with intercropping switchgrass between rows of loblolly pine (Minick et al., 2015; Cacho et al., 2018a). We do not have a clear explanation for this, but Cacho et al. (2018a) surmised that there could be a species-induced effects of planting switchgrass in a formerly loblolly pine-dominated vegetation on the population of nitrifiers along with impacts of varying management practices and environmental conditions. One could argue that net nitrification could have been limited by NH_4^+N production since net N_m in SWITCH was consistently lower than PINE (Fig. 3a and b) even though their differences were not significant (Table 3). This is not true, because, while average annual net N_m rates across treatments significantly ($p = 0.048$) increased from 2011 to 2012, mean relative nitrification (the portion of total mineral N as nitrate at the end of incubation) (N_R) in SWITCH slightly decreased from 16% to 14% as opposed to mean N_R in PINE which increased from 33% to 37% (Fig. 3h).

Unlike PINE, SWITCH underwent three consecutive annual herbicide applications with fertilization. Past research on the effects of one-time herbicide application with and without fertilization in loblolly pine stands did not significantly affect NO_3^--N production (Andariese and Vitousek, 1988; Blazier et al., 2005). However, it is uncertain how a complete conversion of managed loblolly pine (a C3-dominated system) into switchgrass monoculture (a C4 species) subjected to a high frequency herbicide application and fertilization is going to impact nitrifiers. Negative impact of herbicide on nitrification has been shown to be accentuated by fertilization even though the overall N mineralization is increasing (e.g. Marsh, 1985). Combined annual application of herbicide and fertilizer during the first 20 years of a loblolly pine stand in Georgia, U.S.A. had the most significant negative impact on soil microbial biomass than when either management was applied separately (Rifai et al., 2010).

It also appeared that nitrifiers in SWITCH were significantly affected by environmental conditions, particularly soil water (SW) content. As discussed previously, treatment effect on SW content was significant ($p = 0.02$), but not on soil TMP ($p = 0.685$). In the field, aeration conditions in SWITCH may not be as favorable for nitrification as with PINE. With optimum environmental conditions in the laboratory, net N_n rates between PINE and SWITCH were not significantly different ($p = 0.061$), but in situ net N_n rate in SWITCH was significantly lower than PINE ($p = 0.006$). While ammonification can still occur at relatively higher rates in saturated conditions (Rodrigo et al., 1997), nitrification declined significantly at SW content above field capacity and can cease completely at near saturation (Alexander, 1991).

There could also be a species-induced impacts on nitrifiers with the introduction of switchgrass, a C4 species into a formerly C3 species dominated system. The ability of grasses, through their root exudates, to negatively impact nitrification have long been known (Munro, 1966), a phenomenon that is recently known as biological nitrification inhibition (BNI) (Subbarao et al., 2006). We could not find any BNI information on switchgrass or PINE to support our hypothesis, but, in general, BNI activity tends to be higher in C4 than C3-species (Subbarao et al., 2007a) and in perennial compared to annual plants (Subbarao et al., 2007b).

Nevertheless, across treatments, our average annual areal values of N_m and N_n which ranged from 62 to 167 kg N·ha^{-1} and 16.45 to 62.98 kg N·ha^{-1}, respectively (Table 4) were within the range of values from past studies in managed loblolly pine sites on the same region (e.g., 22.4–143.2 kg N·ha^{-1} for N_m, Fox et al., 1986; Piatek and Allen, 1999; Li et al., 2003; Gurlevik et al., 2004, and 0.3–69.5 kg N·ha^{-1} for N_n, Li et al., 2003; Gurlevik et al., 2004). Similarly, mean annual NR values (14 to 37%, Fig. 3h) were within the range of typical values found in a loblolly pine forest (8–72%, Gurlevik et al., 2004) and in general, in a forest or grassland (Robertson, 1982).

Table 4
In-situ areal mean net N mineralization (N_m) (kg N·ha^{-1}) and nitrification (N_n) (kg N·ha^{-1}) rates in 2011 and 2012 for all treatments. Values inside parentheses are standard error of the mean.

Treat		2011	2012
PINE	N_m	136.98 (35.71)	167.21 (33.22)
	N_n	56.31 (12.44)	62.98 (9.91)
SWITCH	N_m	62 (41.72)	142.61 (38.82)
	N_n	16.45 (14.54)	30.45 (11.58)
REF	N_m	63.57 (18.93)	127.95 (36.96)
	N_n	32.94 (6.1)	31.99 (8.61)

Table 5
Results (p-values) of type III test of fixed effects ANOVA of net N mineralization (N_m) and net nitrification (N_n) rates, and relative nitrification (N_R) from laboratory incubation.

Source	DF	N_m	N_n	N_R
Treat	1	0.363	0.061	0.151
Time	3	0.583	0.371	0.118
Treat * time	3	0.417	0.485	0.511

5. Conclusions, limitations, and recommendations

Conversion of managed loblolly pine (PINE) into switchgrass monoculture (SWITCH) in poorly drained soil did not significantly impact total soil mineral N availability and its temporal variations during the years when switchgrass is expected to reach potential productivity. Rates of annual total mineral N production per unit area were comparable to the range of values found in similar systems in the region. There is also a potential for minimal offsite water quality impacts since SWITCH significantly reduced nitrate N production. Results indicated that field conditions, particularly higher soil water content in SWITCH significantly decreased net nitrification rate.

This study was conducted only for two years (3rd and 4th year after switchgrass establishment). If it is logistically practical to do and financial support is not limiting, future similar studies should be conducted for four years, from the first to fourth year of switchgrass establishment

in order to have a clear picture on the immediate influence of operations associated with site preparation on soil N availability and dynamics. Most importantly, future research should focus on understanding impacts of management-induced (e.g., repeated and successive annual combined herbicide and fertilizer along with compaction) and species-induced (e.g., biological nitrification inhibition activity) impacts of establishing switchgrass monoculture on previously managed forest systems on nitrifiers to optimize its beneficial impacts on productivity and water quality.

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Appendix A

Table A.1
Bulk density (ρ_b), pH, total carbon (TC) content total nitrogen (TN) content, and carbon-to-nitrogen ratio (C:N) of all treatments at the top 20 cm. Values inside parentheses are standard error of the mean.

Treat	ρ_b[a]	pH	TC[b]	TN[c]	C:N
PINE	0.89 (0.09)	4.01 (0.05)	63.14 (11.45)	2.71 (0.62)	23.97 (1.81)
SWITCH	1.04 (0.10)	4.20 (0.05)	48.75 (11.45)	2.30 (0.62)	22.84 (1.81)
REF	0.95 (0.01)	4.02 (0.03)	60.49 (8.95)	1.94 (0.32)	31.68 (0.69)

[a] $g \cdot cm^{-3}$.
[b] $g\ C \cdot kg\ soil^{-1}$.
[c] $g\ N \cdot kg\ soil^{-1}$.

Table A.2
Results (p-values) of type III test of fixed effects ANOVA of physical and chemical properties between PINE and SWITCH at 20 cm from soil surface.

Parameter	DF	P > F
Bulk density (ρ_b)	1	0.072
pH	1	0.071
Total carbon (TC) content	1	0.424
Total nitrogen (TN) content	1	0.658
Carbon-to-nitrogen ratio (C:N)	1	0.586

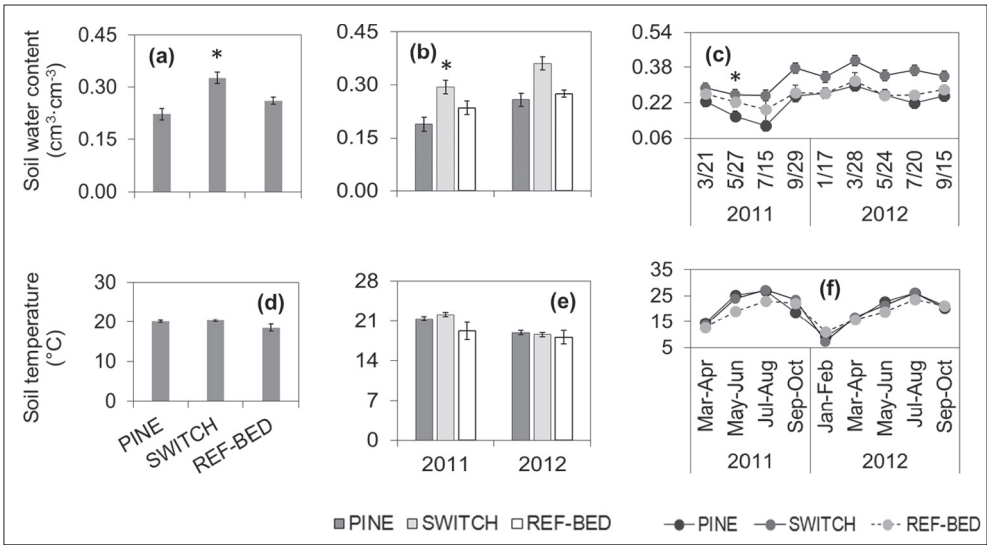

Fig. A.1. Environmental factors; (a) mean soil water content ($cm^3 \cdot cm^{-3}$) at the start of each incubation period and (b) mean soil temperature (°C) during each incubation period at 10 cm from soil surface. Error bars represent standard error of the mean.

Table A.3
Results (p-values) of type III test of fixed effects ANOVA of mean soil water (SW) content ($cm^3 \cdot cm^{-3}$) and mean soil temperature (TMP) (°C) during each incubation period between PINE and SWITCH from in-situ incubation.

Source	DF	SW	TMP
Treat	1	0.02	0.685
Year	1	0.018	0.001
Treat * year	1	0.415	0.576

Table A.3 (*continued*)

Source	DF	SW	TMP
Time (year)	7	0.066	<0.0001
Treat * time (year)	7	0.275	0.797

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